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Articles

Synthesis and Evaluation of Novel Rhodacyanine Dyes That Exhibit Antitumor Activity

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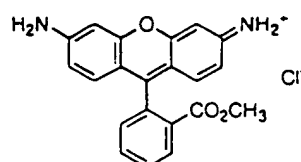
Rhodacyanine dyes and several analogous delocalized lipophilic cations (DLCs) were synthesized and evaluated as novel antitumor agents. Rhodacyanine dye consists of two heteroaromatic rings such as thiazoles at both termini of the conjugate systems and 4-oxothiazolidine (rhodanine) in the middle of it. Compounds with such a unique double-conjugate structure were found to inhibit the growth of several tumor cell lines, such as colon carcinoma CX-1, and to exhibit relatively low toxicity against normal kidney cell line CV-1 (e.g., $IC_{50}(CX-1) = 50$ nM, $IC_{50}(CV-1) = 17.3$ μ M; selectivity index = 346 for compound 5). These compounds were also found to be efficacious in the tumor-bearing nude mice model (e.g., against human melanoma LOX; T/C (%) = 168 for compound 5). Structural modifications on rhodacyanine, including deletion of a heteroaromatic ring involved in the merocyanine conjugate system and replacement of rhodanine with a structurally related moiety such as 4-oxoimidazolidine or 4-oxo-1,3-dithiolane, resulted in a loss of the selectivity and/or the activity. Our current structure-activity studies imply that the double-conjugate system with a rhodanine moiety is essential for the selective activity of rhodacyanine dyes, and we find this class of compounds as unique antitumor agent candidates.

Introduction

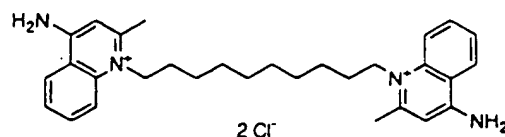
Mitochondria carry out most cellular oxidations and produce the animal cell's ATP. In mitochondria the energy available from combining oxygen with reactive electrons carried by NADH pumps out protons from the inner membrane, and the energy is thus stored in the electrochemical proton gradient which consists of a membrane potential and a pH gradient. The resultant transmembrane gradient is in turn used to synthesize ATP. The membrane potential has been monitored by organic compounds known as membrane potential sensitive probes.¹⁻³ These probes, most of which are the positively charged organic compounds, are taken into cells in response to the high negative charge in the mitochondria, where they can be toxic preferentially. Since the membrane potential of the mitochondria in tumor cells is higher than that of normal cells, these compounds are accumulated in the tumor mitochondria.⁴⁻⁶ Therefore, cytotoxic π electron-delocalized lipophilic cations (DLCs) are proposed to kill tumor cells selectively, and DLCs including rhodamine 123,⁴⁻⁷ dequalinium,⁸ thiacyanocyanines,^{9,10} and thiopyrylium AA-1 (Chart 1)¹¹ have been explored as potential antitumor drugs. In spite of high potential as antitumor agents, none of them have met the criteria for clinical development, such as water solubility, stability, toxicity, and pharmacokinetics.

In an effort to find novel DLCs, we have screened a wide variety of DLCs from the compound library at Fuji

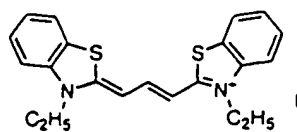
Chart 1



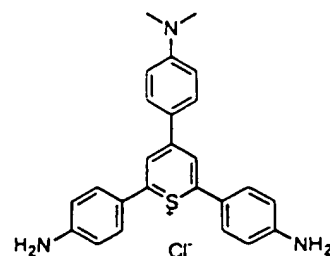
Rhodamine 123



Dequalinium



Thiacyanocyanine (S23)



Thiopyrylium (AA-1)

Photo Film Co., Ltd. which was developed for photographic systems. Rhodacyanine dyes that were originally studied as silver halide sensitizers showed high inhibitory effect *in vitro* on the growth of tumor cells. In this paper we focus on synthesis and evaluation of

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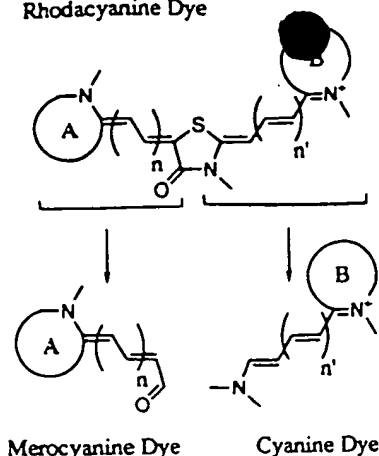
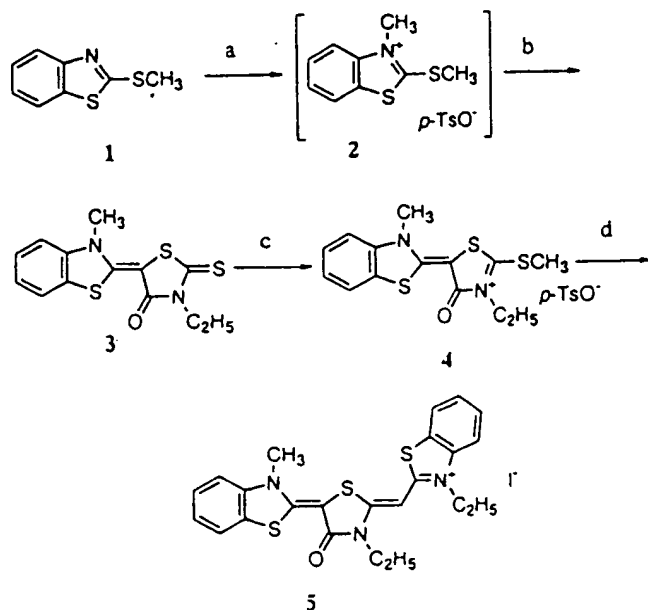


Figure 1. General formula of rhodacyanine dye and its component parts: merocyanine dye and cyanine dye.

Scheme 1^a



^a $p\text{-TsO}^- = p\text{-CH}_3\text{-C}_6\text{H}_4\text{-SO}_3^-$

^a Reagents: (a) methyl *p*-toluenesulfonate/anisole; (b) 3-ethyl-4-oxothiazolidine-2-thione/ NEt_3/MeCN ; (c) methyl *p*-toluenesulfonate/DMF; (d) 3-ethyl-2-methylbenzothiazolium iodide/ NEt_3/MeCN .

novel rhodacyanine dyes and their analogs to clarify structure-activity relationships.

Chemistry

Rhodacyanine dye consists of three rings, two heteroaromatic rings and a central 4-oxothiazolidine (rhodanine), as represented in Figure 1. At the rhodanine moiety, two dye conjugate systems, neutral merocyanine with heteroaromatic ring A and cationic cyanine with heteroaromatic ring B, are integrated. In the present study, we synthesized two types of rhodacyanine dyes, ($n = 0, n' = 0$) and ($n = 1, n' = 0$) in Figure 1, according to the reported procedures¹² with some modifications. Typical synthesis examples are illustrated in Schemes 1 and 2, respectively. In both cases, merocyanine dyes (3 and 8) were synthesized first followed by the conjugation with cyanine units.

N-Methylation of 2-(methylthio)benzothiazole (1) using methyl *p*-toluenesulfonate gave 2, which was con-

densed with 3-ethyl-4-oxothiazolidine-2-thione to give merocyanine dye 3. Methylation of 3 was followed by the reaction of 3-ethyl-2-methylbenzothiazolium iodide in the presence of triethylamine to give a rhodacyanine dye (5). Other rhodacyanine dyes ($n = 0, n' = 0$) were also synthesized in an analogous manner, and the results are summarized in Table 1.

3-Ethyl-4-oxothiazolidine-2-thione (6) was reacted with 1,3-diaza-1,3-diphenylpropene and then with acetic anhydride to give compound 7, which was condensed with 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium iodide to give merocyanine dye 8. A rhodacyanine dye (10) was synthesized from merocyanine intermediate 8 under similar conditions as those for the synthesis of 5 from 3. Other rhodacyanine ($n = 1, n' = 0$) dyes were also synthesized in an analogous manner, and the results are summarized in Table 1.

Because the rhodanine moiety is conjugated with two double bonds, there are several possible geometrical isomers around this moiety. However, our NMR studies suggested that these rhodacyanine dyes exist as a single isomer. As illustrated in Figures 2 and 3, X-ray crystallographic studies for compounds 32 and 33 revealed that in the merocyanine unit *N*-substituents were situated on the opposite side of methine carbons, while in the cyanine unit *N*-substituents were sitting on the same side for both cases. As for the geometrical isomerism of the merocyanine moiety of 33, it was determined to be the *s-trans* configuration (Figure 3).

For structure-activity studies, several DLC compounds analogous to rhodacyanines, including analogs whose heteroaromatic ring A was replaced with other moieties (13a,b, 14, 15, 23) and those whose rhodanine moiety was substituted with other structurally related moieties (20, 26, 28, 30), were synthesized. The results are summarized in Tables 2 and 3.

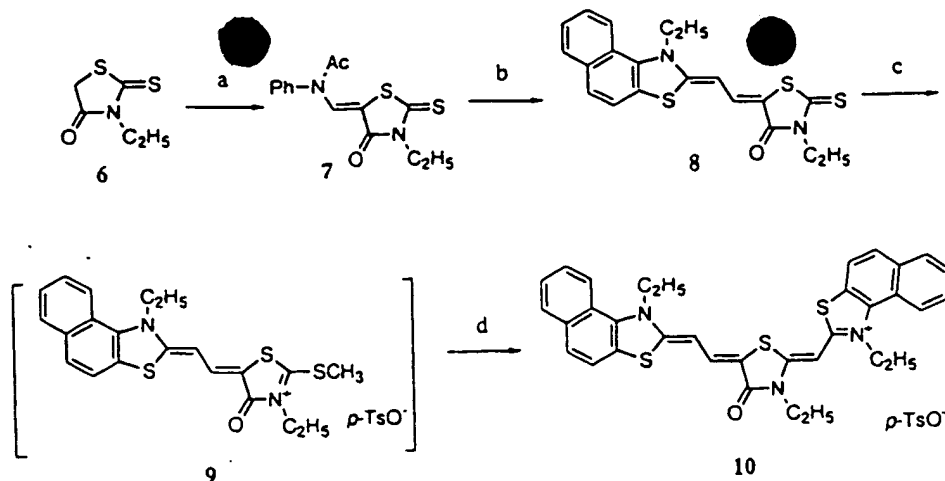
Synthesis of cyanine dye 13b was accomplished by the reaction of 3-ethyl-2-methylbenzothiazolium iodide (11) with ethyl thiocyanate followed by the reaction of bromoacetic acid as illustrated in Scheme 3.¹³ Treatment of 11 with phenyl thiocyanate gave *N*-phenyl derivative 13a, which was converted to 14 and 15 by the reaction of 1,3-diaza-1,3-diphenylpropene and 1,5-diaza-1,5-diphenyl-1,3-pentadiene, respectively.

A rhodanine analog (20) was synthesized according to the reported procedures as illustrated in Scheme 4.^{14,15} Carbodithioate 16¹⁴ was treated with triethyl orthoformate and then 3-ethyl-2-methylbenzoxazolium iodide to give merocyanine dye 18, which was further converted to the target compound 20 under similar conditions as those for the synthesis of rhodacyanine dye 10.

Other analogs of the rhodanine moiety (26 and 28) containing 4-oxoimidazolidine were synthesized by reported procedures similar to those for the synthesis of the corresponding rhodacyanine dye.¹⁶ The other rhodanine analog (30) containing 4-oxo-1,3-dithiolane¹⁶ and cyanine dyes (21 and 22)¹⁰ were also synthesized by reported procedures, respectively.

Biological Results and Discussion

In Vitro Clonogenic Assay. The compounds synthesized here were evaluated for their inhibitory effects on the growth of human colon carcinoma cell (CX-1) and toxicity against normal epithelial cell (CV-1). In each



^a Reagents: (a) (i) 1,3-diaza-1,3-diphenylpropene/ligroin, (ii) Ac₂O/NEt₃; (b) 3-ethyl-2-methylnaphthol[1,2-*d*]thiazolium *p*-toluenesulfonate/Ac₂O/NEt₃/MeCN; (c) methyl *p*-toluenesulfonate/DMF/toluene; (d) 3-ethyl-2-methylnaphthol[1,2-*d*]thiazolium *p*-toluenesulfonate/NEt₃/MeCN.

Table 1. Structure of Rhodacyanine Dyes

Compd	a ₁ , a ₂	a ₃	R ₁	n	R ₂	b ₁	b ₂	X ⁻
5	H, H	S	CH ₃	0	C ₂ H ₅	S		Γ AcO ⁻
24	H, H	S	CH ₃	0	Ph	S		<i>p</i> -TsO ⁻
32	H, H	S	CH ₃	0		O		Cl ⁻
10		S	C ₂ H ₅	1	C ₂ H ₅	S		<i>p</i> -TsO ⁻
25	H, H	S	C ₂ H ₅	1	Ph	S		Γ
27		S	C ₂ H ₅	1	C ₂ H ₅	S		<i>p</i> -TsO ⁻
29	H, H	O	C ₂ H ₅	1	CH ₃	S		Γ
33	CF ₃ , H	S	C ₂ H ₅	1	C ₂ H ₅	S	H, CH ₃	Γ

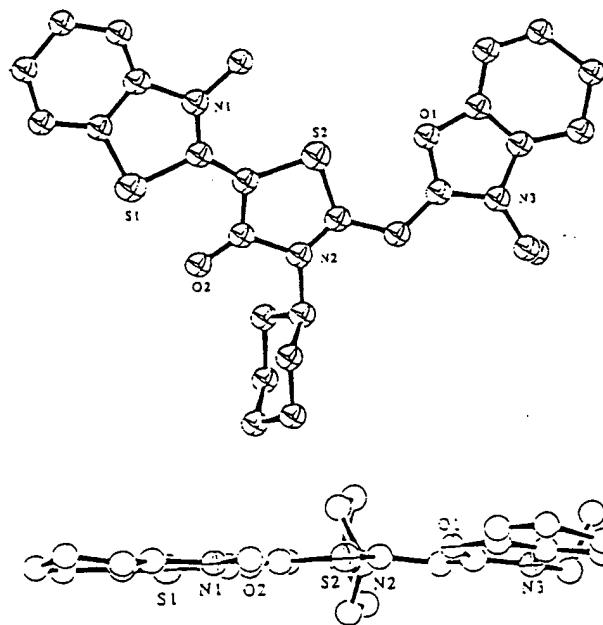


Figure 2. X-ray-determined structure of rhodacyanine dye 32.

case, the cells were incubated with a compound for 24 h and then in compound-free medium for 2 weeks. The number of colonies was measured by a crystal violet-staining method, and the results are expressed as the IC₅₀ value.

Cyanine dyes such as thiacyanine (S23)⁹ and other DLCs^{4-8,11} were reported to exhibit selective activity against carcinoma cells. In this study two cyanine dyes with different methine lengths, 21 (S23) and 22, showed high inhibitory effect on CX-1 with IC₅₀ values of 60 nM (Table 4). As a part of structure modifications of cyanine dyes in an attempt to find a novel class of DLCs with higher activity and selectivity, a methine unit of the cyanine dyes was replaced with a

rhodanine moiety. The rhodacyanine dyes thus synthesized were found to be equipotent (5) or 1.6 times more potent (10) to their cyanine counterparts as depicted in Table 4. Another interesting finding about rhodacyanines in Table 4 is their low toxicity against CV-1 and the resulting high selectivity defined by IC₅₀(CV-1)/IC₅₀(CX-1): they were more selective than the corresponding cyanines by 17-fold (5 vs 21) and 109-fold (10 vs 22).

To understand why rhodacyanine dyes have such a high activity specifically against tumor cells, our first structure-activity study focused on deletion or replacement of the heteroaromatic ring of the merocyanine conjugate system (ring A in Figure 1). Simple deletion of the *N*-ethylbenzothiazolidene group of rhodacyanine 5 led to a decrease of the activity by 26-fold (5 vs 13b in Table 5). Replacement with a cyclohexylidene group (23) resulted in further loss of activity. While compounds 13b and 23 still retain some moderate activities, it may be due to the delocalized cationic cyanine

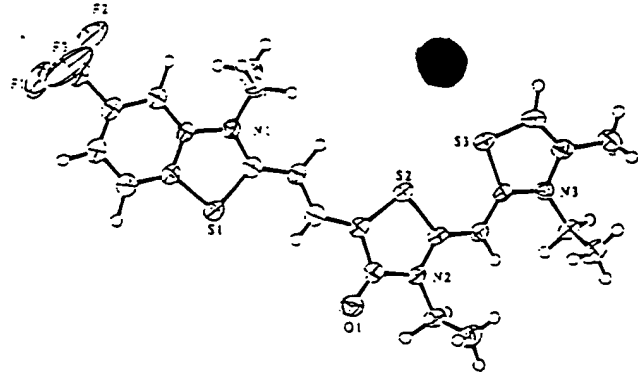


Figure 3. X-ray-determined structure of rhodacyanine dye 33.

Table 2. Structure of Rhodacyanine Dye Analogs

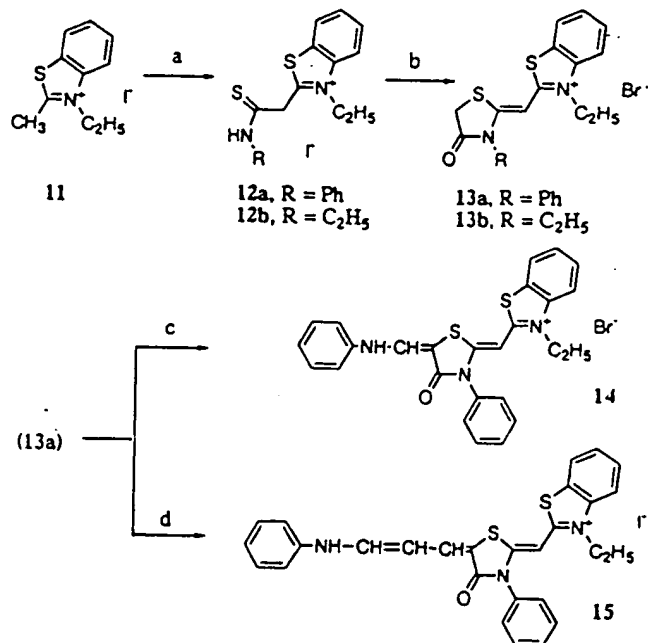
Compd	R ₁ , R ₂	R ₃	X ⁻
13a	H, H	Ph	Br ⁻
13b	H, H	C ₂ H ₅	Br ⁻
14		Ph	Br ⁻
15		Ph	I ⁻
23		C ₂ H ₅	<i>p</i> -TsO ⁻

conjugate system (between rhodanine and ring B) in these compounds. The fact that relatively low selectivity of these compounds (30 for 13b, 9 for 23) compared to a rhodacyanine (346 for 5) are within the same range for cyanine dyes 21 and 22 in Table 4 supports this hypothesis. The results of *N*-phenyl derivatives were quite similar to those of *N*-ethyl derivatives (Table 5). Deletion (13a) or replacement (14, 15) of the benzothiazolidene moiety of rhodacyanine 24 made the activity and selectivity as low as those for 13b and 23. In contrast, elongation of the methine unit between the A ring and rhodanine moiety in rhodacyanine dye (from *n* = 0 to *n* = 1 in Figure 1) affected the quite selective activity of 24 only in a moderate way, and the resultant

Table 3. Structure of Rhodacyanine Dye Analogs

Compd	a ₁ , a ₂	a ₃	R ₁	n	C	b ₁ , b ₂	X ⁻
20	H, H	O	C ₂ H ₅	1		H, H	I ⁻
26	H, H	S	CH ₃	0		H, H	I ⁻
28		S	C ₂ H ₅	1			I ⁻
30	H, H	O	C ₂ H ₅	1		H, H	I ⁻

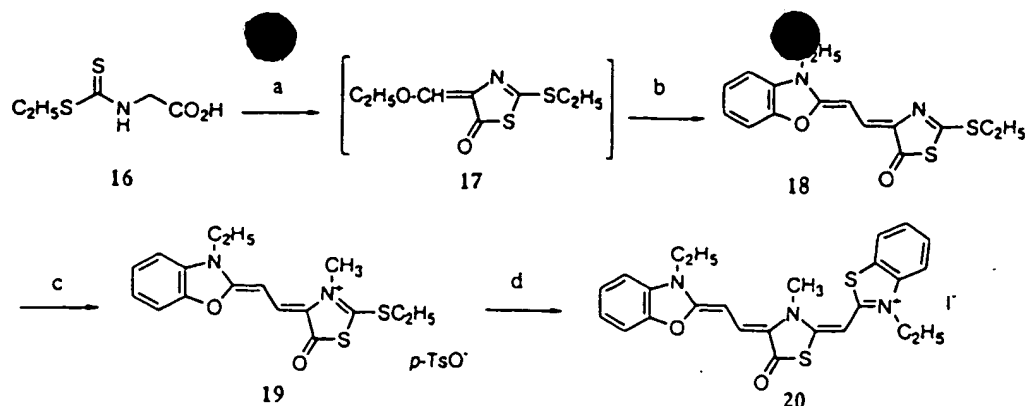
Scheme 3^a



^a Reagents: (a) 12a—phenyl thiocyanate/NaH/THF, 12b—ethyl thiocyanate/pyridine/NEt₃; (b) 13a—bromoacetic acid/1-BuOH, 13b—bromoacetic acid/acetic acid; (c) 1,3-diphenylpropane-1,3-dione/ethylene glycol; (d) 1,5-diphenyl-1,3-pentadiene·HCl salt/NEt₃/NaI/MeOH.

compound 25 showed high activity and selectivity (IC₅₀ against CX-1 = 60 nM, selectivity = 50).

An attempt to measure the IC₅₀ values for merocyanine dyes such as 3 failed due to their poor solubility. In our preliminary experiments, however, they did not exhibit any appreciable activity at 3 μM. A comparison of 3 (merocyanine), 5 (rhodacyanine), and 13b (cyanine), together with other results in Table 5, led us to the hypothesis that the merocyanine conjugate system in rhodacyanine dyes enhanced moderate activity which



^a Reagents: (a) $\text{CH}(\text{OEt})_3/\text{Ac}_2\text{O}$; (b) 3-ethyl-2-methylbenzoxazolium iodide/ NEt_3/EtOH ; (c) methyl *p*-toluenesulfonate; (d) 3-ethyl-2-methylbenzothiazolium iodide-HCl salt/ NEt_3/EtOH .

Table 4. Clonogenic Assay of Rhodacyanine Dyes and Cyanine Dyes on Human Colon Carcinoma (CX-1) and Monkey Normal Kidney Epithelial Cells (CV-1)

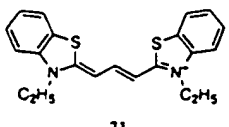
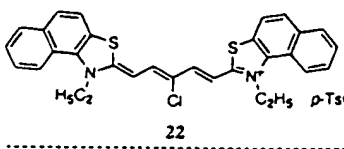
Compd	IC ₅₀ (μM)		Ratio
	CX-1	CV-1	
	0.06	1.2	20
	0.06	0.1	2
5	0.05	17.3	346
10	0.03	6.5	217

Table 5. Clonogenic Assay of Rhodacyanine Dyes and Their Cyanine Dye Analogs

compd	IC ₅₀ (μM)		ratio CV-1/CX-1
	CX-1	CV-1	
5	0.05	17.3	346
24	0.01	2.2	220
25	0.06	3.0	50
13a	2.3	46.1	20
13b	1.3	38.9	30
14	1.5	1.5	1
15	0.8	9.8	12
23	1.8	16.2	9

arised from the cationic cyanine conjugate system in a selective manner.

To obtain further insights into the role of the merocyanine conjugate system, we modified the structure of the rhodanine moiety. Replacement with 4-oxoimidazolidine resulted in a decrease of the activity against CX-1 cells (5 vs 26, 27 vs 28 in Table 6). Compound 28 was more toxic against CV-1 than the corresponding rhodacyanine 27 by 33-fold, resulting in the marked decrease of the selectivity (66-fold). Analysis of Table 6 reveals that an analog with the 4-oxo-1,3-dithiolan moiety (30) exhibited further decreased activity (>50 times), while substitution with 5-oxothiazoline (20) made the activity only one-half that of the parent compound (29). Thus, our structure-activity studies so far concluded that the rhodanine moiety is indispensable for the quite high and selective activity for tumor cells.

Table 6. Clonogenic Assay of Rhodacyanine Dyes and Their Analogs of the 4-Oxothiazolidine (Rhodanine) Moiety

compd	IC ₅₀ (μM)		ratio CV-1/CX-1
	CX-1	CV-1	
5	0.05	17.3	346
27	0.05	26.2	524
29	0.03	1.7	57
20	0.07	3.4	49
26	1.3	25.4	20
28	0.1	0.8	8
30	>1.7	3.4	<2

Table 7. Clonogenic Assay on Four Human Cancer Cell Lines^a

compd	anion	IC ₅₀ (μM)			
		EJ	LOX	MFC-7	CRL1420
5	I ⁻	0.07	0.02	0.09	0.05
31	AcO ⁻	0.06	0.01	0.4	0.06
24	<i>p</i> -TsO ⁻	0.04	0.04	0.06	0.04
25	I ⁻	0.4	0.04	0.04	0.07

^a EJ, bladder carcinoma; LOX, melanoma; MCF-7, breast carcinoma; CRL1420, pancreatic carcinoma.

Our physical chemistry studies including X-ray crystallography, absorption and NMR spectra, and molecular orbital calculations revealed that all three rings (A, B, rhodanine) were almost coplanar and that π electrons delocalized throughout the molecule. This electron delocalization across the two dye conjugate systems was partly indicated from a marked bathochromic shift of rhodacyanine 5 (λ_{max} : 500.0 nm in methanol) compared to the partial structured merocyanine 3 (λ_{max} : 428.0 nm in methanol) and cyanine 13b (λ_{max} : 390.4 nm in methanol). A molecular orbital calculation suggested that the *p* orbital of rhodanine's sulfur atom was involved in such an electron delocalization, which was thought to be a key factor for the selective activity of rhodacyanine dyes against tumor cells. Further studies concerning the relationship between physical property and activity are in progress.

Further *in vitro* studies of rhodacyanines 5, 31, 24, and 25 were conducted using four human tumor cell lines: EJ (human bladder carcinoma), LOX (human melanoma), MCF-7 (human breast carcinoma), and CRL-1420 (human pancreatic carcinoma). As summarized in Table 7, all the compounds showed high inhibitory activities against the cell lines with IC₅₀ values of 10–90 nM, suggesting that rhodacyanine dyes are potential medications for a wide spectrum of tumors. The highly water-soluble compound 31 (solubility > 10

compd	treatment	in vivo activity		
		T/C ^a (%)	OVCAIII	TI ^b (%) CX-1
5	at 5 mg/kg ip on days 1, 5, 8, 12, and 15	168		
31	at 5 mg/kg ip on days 1, 3, 7, 10, and 16		180	
	at 2 mg/kg ip on days 1, 2, 4, 5, 7, 8, and 10	156		
	at 4 mg/kg ip on days 1, 2, 4, 5, 7, 8, and 10	163		
24	at 5 mg/kg ip on days 1, 5, 8, and 12	183		
	at 5 mg/kg ip on days 1, 3, 7, 10, and 16		>300	
	at 5 mg/kg ip on days 1, 5, and 7			42
25	at 1.5 mg/kg ip on days 1, 3, 4, 7, and 9	256		
				<0.05

^a T/C represents the ratio of the median survival time of drug-treated to control, untreated tumor-bearing mice, expressed as a percentage. Each group consisted of five nude mice. ^b TI represents tumor inhibition ratio, which was evaluated at day 11 and calculated as follows: inhibition ratio (%) = $(A - B)/A \times 100$, where A is the average tumor weight in the control group and B is that in the treated group. Each group consisted of five nude mice. ^c The statistical significance of difference between the control and the drug-treated group was determined by applying the Bartlett's, Dunnett's, and Scheffé's test.

mg/mL), which has an acetate anion and was prepared from the corresponding less soluble iodide 5 (solubility < 0.1 mg/mL) using an anion-exchange method, has similar IC₅₀ values to those for 5, which implies that the cation dye is the active component while the counteranion has little influence upon activities.

In Vivo Antitumor Activities. Rhodacyanine dyes 5, 31, 24, and 25 were evaluated for their inhibitory activities against the growth of human cell lines in the nude mice model. After intraperitoneal (ip) implantation of 2×10^6 human melanoma LOX cells (to which the compounds exhibited the highest activity in Table 7), male Swiss nu/nu mice had a median survival time of 24 days. In contrast, mice that received ip administration of compound 5 at 5 mg/kg on days 1, 5, 8, 12, and 15 showed a median survival time of 40 days with tumor/control (T/C) = 168% (Table 8). Administration of other compounds with appropriately determined doses and schedules made the survival period similar or even longer: T/C = 156% (2 mg/kg) or 163% (4 mg/kg) for 31, 183% for 24, 256% for 25. Compounds 5 and 24 were also tested against human ovarian carcinoma OVCARIII, and both were found to be quite efficacious (T/C = 180% for 5, >300% for 24), and compound 24 in the human colon carcinoma CX-1 nude mice model displayed marked inhibition of tumor growth (tumor inhibition ratio (TI) = 42%). Thus simple anion exchange had little influence upon *in vivo* antitumor efficacy and was a useful method to increase the water solubility which was an important criterion for clinical use. Any significant body weight loss was not observed in these nude mice models, and also the acute toxicity (LD₅₀, ip administration into ICR mice) of compound 31 observed was over 30 mg/kg.

Conclusion

The results described in this study demonstrated that rhodacyanine dyes exhibited selective inhibitory activities *in vitro* against the growth of several human cell lines over CV-1, an indicator cell for normal epithelial cells. They also displayed high-level efficacy against LOX and OVCARIII *in vivo*, with low toxicity. Structure-activity studies indicated that rhodanine was an indispensable moiety for the antitumor activity of rhodacyanines, which was markedly improved from those of cyanines or other DLCs. This class of compounds is expected to be a novel antitumor agent and is being

subjected to further extensive structure-activity studies, results of which will be presented elsewhere.

Experimental Section

The ¹H-NMR spectra were recorded on a Bruker AMX-600, ARX-300, or AC-200 spectrometer with tetramethylsilane as internal standard. Chemical shifts are given in ppm, coupling constants are in hertz, and splitting patterns are designated as follows: s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets. Ultraviolet-visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-260 spectrophotometer. Fast atom bombardment (FAB) mass spectra were determined with a JEOL DX303 mass spectrometer, and high-resolution mass spectrometry (HRMS) was recorded on a JEOL SX-102A mass spectrometer; electron ionization (EI) mass spectra were determined with a JEOL JMS-D300 instrument. Elemental analyses were performed on Yanagimoto MT-3 and Dionex 2000i/SP instruments, and the results (C, H, N) were within ±0.4% of theoretical values unless indicated otherwise. Correct elemental analyses for most of the compounds could only be obtained by factoring in partial hydration of these organic salts.

3-Ethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidine-2-thione (3). A mixture of 2-(methylthio)benzothiazole (1) (40.0 g, 220 mmol), methyl *p*-toluenesulfonate (61.5 g, 330 mmol), and anisole (56 mL) was stirred at 120–136 °C for 4 h. After the mixture was cooled to room temperature, 3-ethyl-4-oxothiazolidine-2-thione (6) (35.0 g, 220 mmol) and acetonitrile (800 mL) were added. To this mixture was added triethylamine (36.4 g, 360 mmol) dropwise under 15 °C with constant stirring and cooling, and the resulting mixture was stirred at 10 °C for 4 h. The yellow precipitate was collected and washed with acetonitrile (40 mL) and then with methanol (140 mL). The crude product thus obtained was suspended in acetone (210 mL) and methanol (420 mL), and the mixture was stirred under reflux for 15 min. After cooling to 25 °C, the precipitate was collected and washed with methanol (140 mL) to give 3 (59.0 g, 87.0%) as yellow crystals: UV-vis (MeOH) λ_{\max} 428.0 nm (ϵ 6.62 \times 10⁴); ¹H-NMR (DMSO-*d*₆) δ 1.20 (t, *J* = 7.2 Hz, 3H), 3.98 (s, 3H), 4.10 (q, *J* = 7.2 Hz, 2H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.0 Hz, 1H); MS (EI) *m/z* 308. Anal. (C₁₃H₁₂N₂OS₂) C, H, N, S.

3-Ethyl-5-(3-methylbenzothiazolin-2-ylidene)-2-(methylthio)-4-oxothiazolinium *p*-Toluenesulfonate (4). A mixture of 3 (58.0 g, 188 mmol), methyl *p*-toluenesulfonate (105.0 g, 564 mmol), and *N,N*-dimethylformamide (58 mL) was stirred at 130–145 °C for 2.5 h. After the mixture cooled to 95 °C, acetone (500 mL) was added. The mixture was further cooled to 25 °C with constant stirring, and the precipitate formed was collected and washed with acetone (150 mL). The crude product thus obtained was suspended in acetone (400 mL), and the mixture was stirred under reflux for 15 min. After cooling to 25 °C, the precipitate was collected and washed with acetone

(150 mL) to give 4 (86.5 g, 93.0%) as orange crystals: UV-vis (MeOH) λ_{max} 420.7 nm (ϵ 3.68×10^4); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.33 (t, J = 7.2 Hz, 3H), 2.27 (s, 3H), 3.05 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.24 (s, 3H), 7.10 (d, J = 8.9 Hz, 2H), 7.46 (d, J = 8.9 Hz, 2H), 7.52 (dd, J = 8.0, 8.0 Hz, 1H), 7.70 (dd, J = 8.0, 8.0 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H); MS (FAB⁺, glycerine) for $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}_3\text{S}$ m/z 323, (FAB⁻, triethanolamine) for $\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}_3\text{S}$ m/z 171. Anal. ($\text{C}_{14}\text{H}_{15}\text{N}_2\text{O}_3\text{S} \cdot 0.3\text{H}_2\text{O}$) C, H, N, S.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolidin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]benzothiazolium Iodide (5). To a mixture of 4 (24.7 g, 50 mmol) and 3-ethyl-2-methylbenzothiazolium iodide (15.3 g, 50 mmol) in acetonitrile (250 mL) was added triethylamine (15.2 g, 150 mmol) dropwise at 70 °C, and the mixture was stirred for 1.5 h at the same temperature. To the reaction mixture was added ethyl acetate (250 mL), and the mixture was cooled to 30 °C with constant stirring. The orange precipitate was collected and washed with ethyl acetate (125 mL). The crude product thus obtained was dissolved in methanol (80 mL), and then to this solution was added ethyl acetate (250 mL) at 50 °C with constant stirring. The precipitate was collected and washed with ethyl acetate (110 mL) to give 5 (14.5 g, 50.0%) as orange crystals: UV-vis (MeOH) λ_{max} 500.0 nm (ϵ 7.49×10^4); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.28 (t, J = 7.1 Hz, 3H), 1.38 (t, J = 7.1 Hz, 3H), 4.22 (s, 3H), 4.30 (q, J = 7.2 Hz, 2H), 4.71 (q, J = 7.2 Hz, 2H), 6.69 (s, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.52–7.58 (m, 2H), 7.70–7.79 (m, 2H), 7.94–7.98 (m, 2H), 8.26 (d, J = 7.2 Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for $\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_3\text{S}$ m/z 452, (FAB⁻, glycerine) for m/z 127. Anal. ($\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_3\text{S} \cdot 1.5\text{H}_2\text{O}$) C, H, I, N, S.

5-[(N-Acetyl-N-phenylamino)methylidene]-3-ethyl-4-oxothiazolidine-2-thione (7). To a solution of 3-ethyl-4-oxothiazolidine-2-thione (6) (25.0 g, 155 mmol) in ligroin (145 mL) was added 1,3-diaza-1,3-diphenylpropene (32.5 g, 166 mmol), and the mixture was stirred at 70 °C for 1 h. After cooling to room temperature, the precipitate was collected and washed with acetone (100 mL) to give 3-ethyl-4-oxo-5-[(phenylamino)methylidene]thiazoline-2-thione (45.0 g). This product was mixed with acetic anhydride (110 g, 1080 mmol) and triethylamine (0.18 g, 2 mmol), and the mixture was stirred at 110 °C for 30 min. The reaction mixture was concentrated to about one-half volume under reduced pressure. To this residue was added methanol (225 mL), and the mixture was stirred at 10 °C for 1 h. The precipitate formed was collected and washed with methanol (100 mL) to give 7 (37.0 g, 77.9%) as yellow crystals: $^1\text{H-NMR}$ (DMSO- d_6) δ 1.08 (t, J = 7.2 Hz, 3H), 2.02 (s, 3H), 3.95 (q, J = 7.2 Hz, 2H), 7.47–7.57 (m, 2H), 7.59–7.72 (m, 3H), 8.47 (s, 1H); MS (EI) m/z 306. Anal. ($\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2\text{S}_2$) C, H, N, S.

3-Ethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidine-2-thione (8). A mixture of 7 (29.8 g, 97 mmol), 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium *p*-toluenesulfonate (38.8 g, 97 mmol), and acetic anhydride (14.2 g, 140 mmol) in acetonitrile (1000 mL) was stirred at 50 °C for 1 h. To this was added triethylamine (36.3 g, 359 mmol) at 50 °C, and the mixture was stirred for an additional 4 h at 60 °C. After cooling to 25 °C, the precipitate formed was collected and washed with acetonitrile (250 mL). The crude product thus obtained was suspended in methanol (750 mL), and the mixture was stirred under reflux for 1 h. After cooling to 25 °C, the precipitate was collected to give 8 (27.4 g, 70.7%) as purple-red crystals: $^1\text{H-NMR}$ (DMSO- d_6) δ 1.16 (t, J = 7.2 Hz, 3H), 1.59 (t, J = 7.2 Hz, 3H), 4.04 (q, J = 7.2 Hz, 2H), 4.66 (q, J = 7.2 Hz, 2H), 5.58 (d, J = 13.2 Hz, 1H), 7.54–7.69 (m, 3H), 7.85–7.93 (m, 2H), 8.08 (d, J = 9.0 Hz, 1H), 8.46 (d, J = 9.0 Hz, 1H); MS (HRMS) for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$ calcd 398.0581, found 398.0572. Anal. ($\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$) C, H, N: calcd, 24.13; found, 24.58.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho[1,2-*d*]thiazolium *p*-Toluenesulfonate (10). A mixture of 8 (14.5 g, 36 mmol) and methyl *p*-toluenesulfonate (20.2 g, 109 mmol) in *N,N*-dimethylformamide (35 mL) and toluene (13 mL) was stirred at 115 °C for 6 h. To the mixture was added 3-ethyl-2-methylnaphtho[1,2-*d*]thiazolinium *p*-tolu-

enesulfonate (14.7 g, 36 mmol) in acetonitrile (1080 mL). To this was added triethylamine (11.0 g, 109 mmol) at 75 °C, and the resulting mixture was stirred at 75 °C for an additional 1 h and then cooled to 30 °C. The precipitate formed was collected and washed with acetonitrile (300 mL). The crude product thus obtained was suspended in methanol (720 mL), and the mixture was stirred under reflux for 1 h. After cooling to 25 °C, the precipitate was collected and washed with acetonitrile (300 mL). Compound 10 (20.4 g, 74.1%) was obtained as green crystals: UV-vis (MeOH) λ_{max} 620.0 nm (ϵ 1.00×10^5); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.28 (t, J = 7.2 Hz, 3H), 1.65–1.72 (m, 6H), 2.29 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.72 (q, J = 7.2 Hz, 2H), 4.98 (q, J = 7.2 Hz, 2H), 6.02 (d, J = 12.9 Hz, 1H), 6.62 (s, 1H), 7.05–7.82 (m, 12H), 7.97 (d, J = 8.4 Hz, 1H), 8.03–8.12 (m, 2H), 8.30 (d, J = 8.4 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for $\text{C}_{34}\text{H}_{30}\text{N}_3\text{O}_3\text{S}$ m/z 592, (FAB⁻, nitrobenzyl alcohol) for $\text{C}_{34}\text{H}_{30}\text{N}_3\text{O}_3\text{S}$ m/z 171; HRMS (FAB⁺) for $\text{C}_{34}\text{H}_{30}\text{N}_3\text{O}_3\text{S}$ calcd 592.1551, found 592.1561. Anal. ($\text{C}_{34}\text{H}_{30}\text{N}_3\text{O}_3\text{S} \cdot 2.7\text{H}_2\text{O}$) C, N, S; H: calcd, 5.26; found, 4.84.

3-Ethyl-2-[(N-phenylamino)thiocarbonyl]methyl]benzothiazolium Iodide (12a). To a suspension of sodium hydride (36.0 g, 1.5 mol) in tetrahydrofuran (1.1 L) was added 3-ethyl-2-methylbenzothiazolium iodide (11) (154.1 g, 0.5 mol) in a small portion at room temperature. To this was added dropwise a solution of phenyl thiocyanate (68.9 g, 0.5 mol) in tetrahydrofuran over a period of 1 h at 20–30 °C, and the resulting mixture was stirred under reflux for 30 min. The solvent was evaporated, and to the residue was added water (500 mL) dropwise under 10 °C. The precipitate formed was collected and washed with water (500 mL) to give 12a (131.3 g, 84.0%) as yellow crystals. Compound 12a was used for the next step without further purification.

3-Ethyl-2-[(3-phenyl-4-oxothiazolidin-2-ylidene)methyl]benzothiazolium Bromide (13a). A mixture of compound 12a (11.0 g, 35 mmol) and bromoacetic acid (11.0 g, 79 mmol) in 1-butanol (22 mL) was stirred at 100 °C for 10 min. After cooling to room temperature, to the mixture was added diethyl ether (100 mL). The precipitate formed was collected and washed with diethyl ether (50 mL) to give the crude product, which was recrystallized from methanol (75 mL) to give 13a (10.9 g, 73.0%) as brown crystals: UV-vis (MeOH) λ_{max} 377.8 nm (ϵ 3.70×10^4); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.13 (t, J = 7.2 Hz, 3H), 4.29 (q, J = 7.2 Hz, 2H), 4.58 (s, 2H), 5.97 (s, 1H), 7.52 (dd, J = 7.5, 1.5 Hz, 2H), 7.63–7.75 (m, 4H), 7.79 (t, J = 7.2 Hz, 1H), 8.11 (d, J = 7.9 Hz, 1H), 8.41 (d, J = 7.9 Hz, 1H); MS (FAB⁺, glycerine) for $\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_2\text{S}_2$ m/z 353, (FAB⁻, glycerine) for Br m/z 79, 81. Anal. ($\text{C}_{19}\text{H}_{17}\text{BrN}_2\text{O}_2\text{S}_2 \cdot 1.1\text{H}_2\text{O}$) C, H, Br, N, S.

3-Ethyl-2-[(N-ethylamino)thiocarbonyl]methyl]benzothiazolium Iodide (12b). A solution of 3-ethyl-2-methylbenzothiazolium iodide (11) (36.0 g, 118 mmol) and triethylamine (4.4 g, 43 mmol) in pyridine (60 mL) was stirred at 110 °C for 20 min, and then the reaction mixture was poured into water (2000 mL). The precipitate formed was collected to give 12b (19.0 g, 60.9%) as purple crystals. Compound 12a was used for the next step without further purification.

3-Ethyl-2-[(3-ethyl-4-oxothiazolidin-2-ylidene)methyl]benzothiazolium Bromide (13b). A solution of compound 12b (6.0 g, 23 mmol) and bromoacetic acid (6.0 g, 43 mmol) in acetic acid (20 mL) was stirred at 90 °C for 5 min. After the mixture cooled to room temperature, diethyl ether (30 mL) was added. The precipitate formed was collected and washed with diethyl ether (20 mL) to give the crude product, which was recrystallized from methanol (350 mL) to give 13b (5.6 g, 77.1%) as yellow crystals: UV-vis (MeOH) λ_{max} 390.4 nm (ϵ 4.20×10^4); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.29 (t, J = 7.2 Hz, 3H), 1.39 (t, J = 7.2 Hz, 3H), 4.09 (q, J = 7.2 Hz, 2H), 4.45 (s, 2H), 4.86 (q, J = 7.2 Hz, 2H), 6.82 (s, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.82 (t, J = 7.5 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.39 (d, J = 7.9 Hz, 1H); MS (FAB⁺, glycerine) for $\text{C}_{15}\text{H}_{17}\text{N}_2\text{O}_2\text{S}_2$ m/z 305, (FAB⁻, glycerine) for Br m/z 79, 81. Anal. ($\text{C}_{15}\text{H}_{17}\text{BrN}_2\text{O}_2\text{S}_2 \cdot 0.7\text{H}_2\text{O}$) C, H, Br, N, S.

3-Ethyl-2-[(3-phenyl-5-[(N-phenylamino)methylidene]-4-oxothiazolidin-2-ylidene)methyl]benzothiazolium Bromide (14). A mixture of 13a (13.0 g, 30 mmol) and 1,3-diaza-

ml) was stirred at 80 °C for 30 min. After cooling to room temperature, to this mixture was added diethyl ether (30 mL), and the precipitate formed was collected. The crude product thus obtained was suspended in 2-propanol (60 mL) and stirred at room temperature for 10 min. The precipitate was collected and washed with isopropyl alcohol (30 mL) to give 14 (18.0 g, quantitative) as yellow crystals: UV-vis (MeOH) λ_{max} 480.3 nm (ϵ 6.26 $\times 10^4$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.14 (t, J = 7.2 Hz, 3H), 4.26 (q, J = 7.2 Hz, 2H), 6.00 (s, 1H), 7.12–7.21 (m, 1H), 7.40–7.51 (m, 3H), 7.53–7.82 (m, 7H), 8.06 (d, J = 7.5 Hz, 1H), 8.36 (d, J = 7.5 Hz, 1H), 8.46 (t, J = 7.5 Hz, 1H), 11.01 (d, J = 13.2 Hz, 1H); MS (FAB $^+$, glycerine) for $\text{C}_{26}\text{H}_{22}\text{N}_3\text{O}_2$ m/z 456, (FAB $^-$, nitrobenzyl alcohol) for Br m/z 79, 81. Anal. ($\text{C}_{26}\text{H}_{22}\text{BrN}_3\text{O}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, Br, N, S.

3-Ethyl-2-[[3-phenyl-5-(*N*-phenylamino)-2-propenylidene]-4-oxothiazolidin-2-ylidene]methyl]benzothiazolium Iodide (15). A mixture of 13a (8.7 g, 20 mmol), 1,5-diaza-1,5-diphenyl-1,3-pentadiene hydrochloride salt (25.9 g, 100 mmol), and triethylamine (13.0 g, 128 mmol) in methanol (170 mL) was stirred at 50 °C for 20 min. After the mixture cooled to room temperature, a solution of sodium iodide (9.0 g, 60 mmol) in methanol (45 mL) was added. The precipitate formed was collected to give the crude product, which was suspended in ethanol (160 mL) and stirred at 50 °C for 20 min. The precipitate was collected and washed with ethanol (30 mL) to give 14 (9.8 g, 80.3%) as dark-green crystals: UV-vis (MeOH) λ_{max} 547.4 nm (ϵ 7.46 $\times 10^4$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.13 (t, J = 7.2 Hz, 3H), 4.22 (q, J = 7.2 Hz, 2H), 5.89 (t, J = 12.0 Hz, 1H), 6.04 (s, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 8.1 Hz, 2H), 7.36 (t, J = 7.5 Hz, 2H), 7.56–7.78 (m, 7H), 7.95–8.06 (m, 2H), 8.30–8.45 (m, 2H), 10.67 (d, J = 12.6 Hz, 1H); MS (FAB $^+$, glycerine) for $\text{C}_{28}\text{H}_{24}\text{N}_3\text{O}_2$ m/z 482, (FAB $^-$, nitrobenzyl alcohol) for I m/z 127. Anal. ($\text{C}_{28}\text{H}_{24}\text{IN}_3\text{O}_2 \cdot 1.0\text{H}_2\text{O}$) C, H, I, N, S.

4-[2-(3-Ethylbenzoxazolin-2-ylidene)ethylidene]-2-(ethylthio)-5-oxothiazoline (18). To a solution of carbodithioate 16 (17.9 g, 0.10 mol) in acetic anhydride (80 mL) was added triethyl orthoformate (21.3 g, 0.14 mol), and the mixture was refluxed 1.5 h. After the acetic anhydride was evaporated, to the residue were added 3-ethyl-2-methylbenzothiazolium iodide (28.9 g, 0.10 mol) in ethanol (80 mL) and then triethylamine (18.6 g, 0.18 mol). The resulting mixture was refluxed for 15 min and then cooled to 10 °C. The precipitate formed was collected to give mercyanine dye intermediate 18 (25.9 g, 78.0%). Compound 18 was used for the next step without further purification.

4-[2-(3-Ethylbenzoxazolin-2-ylidene)ethylidene]-2-(ethylthio)-3-methyl-5-oxothiazolium *p*-Toluenesulfonate (19). A mixture of compound 18 (24.9 g, 75 mmol) and methyl *p*-toluenesulfonate (44.7 g, 240 mmol) was stirred at 130 °C for 1 h. After cooling to 40 °C, to the reaction mixture were added ethanol (30 mL) and then diethyl ether (100 mL). The precipitate was collected to give 19 (30.1 g, 77.4%): $^1\text{H-NMR}$ (DMSO- d_6) δ 1.41 (t, J = 7.2 Hz, 6H), 2.29 (s, 3H), 3.92 (s, 3H), 4.35 (q, J = 7.2 Hz, 2H), 7.03–7.13 (m, 3H), 7.47 (d, J = 8.9 Hz, 2H), 7.51–7.61 (m, 2H), 7.81–7.92 (m, 2H), 7.98 (d, J = 13.8 Hz, 1H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_2\text{S}_2$ m/z 347, (FAB $^-$, nitrobenzyl alcohol) for $\text{C}_7\text{H}_7\text{O}_3\text{S}$ m/z 171; HRMS (FAB $^+$) for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_2\text{S}_2$ calcd 347.0888, found 347.0886.

3-Ethyl-2-[[3-methyl-4-[2-(3-ethylbenzoxazolin-2-ylidene)ethylidene]-5-oxothiazolidin-2-ylidenemethyl]benzothiazolium Iodide (20). To a mixture of 19 (10.4 g, 20 mmol) and 3-ethyl-2-methylbenzothiazolium iodide (6.1 g, 20 mmol) in ethanol (65 mL) was added triethylamine (2.6 g, 26 mmol) at room temperature, and the reaction mixture was refluxed for 30 min. After cooling to room temperature, the precipitate was collected and washed with ethanol (30 mL) to yield 20 (7.46 g, 63.3%): UV-vis (MeOH) λ_{max} 574.1 nm (ϵ 2.09 $\times 10^5$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.31 (t, J = 7.2 Hz, 3H), 1.34 (t, J = 7.2 Hz, 3H), 3.83 (s, 3H), 4.15 (q, J = 7.2 Hz, 2H), 4.52 (q, J = 7.2 Hz, 2H), 6.46 (s, 1H), 6.78 (d, J = 13.2 Hz, 1H), 7.29–7.55 (m, 4H), 7.61–7.69 (m, 4H), 7.97 (d, J = 7.0 Hz, 1H); MS (FAB $^+$, glycerine) for $\text{C}_{25}\text{H}_{24}\text{N}_3\text{O}_2\text{S}_2$ m/z 462, (FAB $^-$, glycerine) for I m/z 127. Anal. ($\text{C}_{25}\text{H}_{24}\text{IN}_3\text{O}_2\text{S}_2 \cdot 1\text{H}_2\text{O}$) C, H, I, N, S.

3-Ethyl-2-[3-(3-ethylbenzothiazolin-2-ylidene)-1-propenyl]benzothiazolium Iodide (21). This compound was synthesized from 3-ethylbenzothiazolium iodide and triethyl orthoformate in pyridine according to the reported method:¹⁰ UV-vis (MeOH) λ_{max} 555.8 nm (ϵ 1.56 $\times 10^4$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.35 (t, J = 7.1 Hz, 6H), 4.38 (q, J = 7.1 Hz, 4H), 6.67 (d, J = 12.6 Hz, 2H), 7.41 (t, J = 7.8 Hz, 2H), 7.58 (t, J = 7.8 Hz, 2H), 7.72–7.83 (m, 3H), 8.01 (d, J = 7.8 Hz, 2H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{21}\text{H}_{21}\text{N}_2\text{S}_2$ m/z 365, (FAB $^-$, nitrobenzyl alcohol) for I m/z 127. Anal. ($\text{C}_{21}\text{H}_{21}\text{IN}_2\text{S}_2 \cdot 1.1\text{H}_2\text{O}$) C, H, I, N, S.

3-Ethyl-2-[3-chloro-5-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)-1,3-pentadienyl]naphtho[1,2-*d*]thiazolium *p*-Toluenesulfonate (22). This compound was synthesized from 3-ethylbenzothiazolium iodide and 3-chloro-1,5-diaza-1,5-diphenyl-1,3-pentadiene in pyridine in a manner analogous to the preparation of 21: UV-vis (MeOH) λ_{max} 681.0 nm (ϵ 2.42 $\times 10^5$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.66 (t, J = 7.2 Hz, 6H), 2.29 (s, 3H), 4.73 (q, J = 7.2 Hz, 4H), 6.32 (d, J = 12.9 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 7.55 (t, J = 7.5 Hz, 2H), 7.70 (t, J = 7.5 Hz, 2H), 7.90 (d, J = 12.9 Hz, 2H), 7.97 (d, J = 8.7 Hz, 2H), 8.03–8.11 (m, 4H), 8.42 (d, J = 8.7 Hz, 2H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{31}\text{H}_{26}\text{ClN}_2\text{S}_2$ m/z 525, (FAB $^-$, nitrobenzyl alcohol) for $\text{C}_7\text{H}_7\text{O}_3\text{S}$ m/z 171. Anal. ($\text{C}_{31}\text{H}_{26}\text{ClN}_2\text{O}_3\text{S}_3 \cdot 1\text{H}_2\text{O}$) C, H, Cl, N, S.

2-[[5-Cyclohexylidene-3-ethyl-4-oxothiazolidin-2-ylidene)methyl]-3-ethylbenzothiazolium *p*-Toluenesulfonate (23). This compound was synthesized from 5-cyclohexylidene-3-ethyl-4-oxothiazolidine-2-thione and 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate in a manner analogous to the preparation of rhodacyanine dye 5: UV-vis (MeOH) λ_{max} 425.8 nm (ϵ 5.97 $\times 10^4$); $^1\text{H-NMR}$ (CDCl $_3$) δ 1.29 (t, J = 7.1 Hz, 3H), 1.52 (t, J = 7.1 Hz, 3H), 1.68–1.80 (m, 2H), 1.92–2.10 (m, 4H), 2.31 (s, 3H), 2.47 (t, J = 6.6 Hz, 2H), 3.22 (t, J = 6.6 Hz, 2H), 4.33 (q, J = 7.1 Hz, 2H), 5.06 (q, J = 7.1 Hz, 2H), 7.10 (d, J = 7.8 Hz, 2H), 7.19 (s, 1H), 7.52–7.58 (m, 1H), 7.68 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{21}\text{H}_{25}\text{N}_2\text{O}_2\text{S}_2$ m/z 385, (FAB $^-$, nitrobenzyl alcohol) for $\text{C}_7\text{H}_7\text{O}_3\text{S}$ m/z 171. Anal. ($\text{C}_{28}\text{H}_{32}\text{N}_2\text{O}_4\text{S}_3 \cdot 1\text{H}_2\text{O}$) C, H, N, S.

3-Ethyl-2-[[3-phenyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene)methyl]benzothiazolium *p*-Toluenesulfonate (24). This compound was synthesized from 2-(methylthio)benzothiazole and 4-oxo-3-phenylthiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 5: UV-vis (MeOH) λ_{max} 501.0 nm (ϵ 6.83 $\times 10^4$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.14 (t, J = 7.1 Hz, 3H), 2.28 (s, 3H), 4.20 (q, J = 7.1 Hz, 2H), 4.30 (s, 3H), 5.93 (s, 1H), 7.10 (d, J = 7.8 Hz, 2H), 7.39 (t, J = 8.1 Hz, 1H), 7.48 (d, J = 7.8 Hz, 2H), 7.51–7.62 (m, 4H), 7.66–7.76 (m, 4H), 7.82 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 8.28 (d, J = 7.6 Hz, 1H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{27}\text{H}_{22}\text{N}_3\text{O}_3\text{S}_3$ m/z 500, (FAB $^-$, nitrobenzyl alcohol) for $\text{C}_7\text{H}_7\text{O}_3\text{S}$ m/z 171; HRMS (FAB $^+$) for $\text{C}_{27}\text{H}_{22}\text{N}_3\text{O}_3\text{S}_3$ calcd 500.0925, found 500.0908.

3-Ethyl-2-[[3-phenyl-5-[2-(3-ethylbenzothiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene)methyl]benzothiazolium Iodide (25). This compound was synthesized from 3-ethyl-2-methylbenzothiazolium *p*-toluenesulfonate and 4-oxo-3-phenylthiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 10: UV-vis (MeOH) λ_{max} 595.4 nm (ϵ 1.07 $\times 10^5$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.13 (t, J = 7.1 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H), 4.19 (q, J = 7.1 Hz, 2H), 4.38 (q, J = 7.1 Hz, 2H), 5.99 (s, 1H), 6.18 (d, J = 13.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.45–7.95 (m, 12H), 8.29 (d, J = 7.2 Hz, 1H); MS (FAB $^+$, nitrobenzyl alcohol) for $\text{C}_{30}\text{H}_{26}\text{N}_3\text{O}_3\text{S}_3$ m/z 540, (FAB $^-$, nitrobenzyl alcohol) for I m/z 127. Anal. ($\text{C}_{30}\text{H}_{26}\text{IN}_3\text{O}_3\text{S}_3 \cdot 0.3\text{H}_2\text{O}$) C, H, I, N, S.

2-[[1,3-Diethyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxoimidazolidin-2-ylidene)methyl]-3-ethylbenzothiazolium Iodide (26). This compound was synthesized from 2-(methylthio)benzothiazole and 1,3-diethyl-4-oxoimidazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 5: UV-vis (MeOH) λ_{max} 481.0 nm (ϵ 4.83 $\times 10^4$); $^1\text{H-NMR}$ (DMSO- d_6) δ 1.01 (t, J = 6.9 Hz, 3H), 1.23 (t, J = 7.1 Hz, 3H), 1.35 (t, J = 7.1 Hz, 3H), 3.95 (q, J = 7.2 Hz,

2H), 4.05 (s, 3H), 4.08 (q, $J = 7.2$ Hz, 2H), 4.42 (q, $J = 7.2$ Hz, 2H), 5.88 (s, 1H), 7.36 (t, $J = 8.0$ Hz, 1H), 7.44 (t, $J = 8.0$ Hz, 1H), 7.54 (t, $J = 8.0$ Hz, 1H), 7.49–7.75 (m, 3H), 7.94 (d, $J = 7.5$ Hz, 1H), 8.04 (d, $J = 7.5$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₅H₂₇N₃O₅ m/z 463, (FAB⁺, nitrobenzyl alcohol) for m/z 127. Anal. (C₂₅H₂₇N₃O₅) C, H, I, N, S.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]naphtho[2,1-*d*]thiazolium *p*-Toluenesulfonate (27). This compound was synthesized from 3-ethyl-2-methyl[1,2-*d*]naphthothiazolium *p*-toluenesulfonate and 3-ethyl-4-oxothiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 10: UV-vis (MeOH) λ_{\max} 622.9 nm (ϵ 1.07 $\times 10^4$); ¹H-NMR (DMSO-*d*₆) δ 1.27 (t, $J = 6.9$ Hz, 3H), 1.38 (t, $J = 7.0$ Hz, 3H), 1.72 (t, $J = 6.9$ Hz, 3H), 2.30 (s, 3H), 4.08 (q, $J = 6.9$ Hz, 2H), 4.52–4.70 (m, 4H), 5.94 (d, $J = 13.2$ Hz, 1H), 6.39 (s, 1H), 6.77–6.88 (m, 2H), 7.14 (d, $J = 8.1$ Hz, 2H), 7.35–7.748 (m, 2H), 7.50–7.67 (m, 6H), 7.78–7.95 (m, 5H); MS (FAB⁺, nitrobenzyl alcohol) for C₃₄H₃₀N₃O₅ m/z 592, (FAB⁺, nitrobenzyl alcohol) for C₇H₇O₃S m/z 171; HRMS (FAB⁺) for C₃₄H₃₀N₃O₅ calcd 592.1551, found, 592.1558.

2-[[1,3-Diethyl-5-[2-(3-ethylnaphtho[1,2-*d*]thiazolin-2-ylidene)ethylidene]-4-oxoimidazolidin-2-ylidenemethyl]-3-ethylnaphtho[2,1-*d*]thiazolium Iodide (28). This compound was synthesized from 3-ethyl-2-methyl[1,2-*d*]naphthothiazolium *p*-toluenesulfonate and 1,3-diethyl-4-oxoimidazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 10: UV-vis (MeOH) λ_{\max} 606.3 nm (ϵ 1.04 $\times 10^4$); ¹H-NMR (DMSO-*d*₆) δ 1.23–1.30 (m, 6H), 1.38 (t, $J = 7.2$ Hz, 3H), 1.74 (t, $J = 7.2$ Hz, 3H), 3.95 (q, $J = 7.2$ Hz, 2H), 4.30 (q, $J = 7.2$ Hz, 2H), 4.41 (q, $J = 7.2$ Hz, 2H), 4.69 (t, $J = 7.2$ Hz, 2H), 5.77 (s, 1H), 7.50–7.58 (m, 3H), 7.62–7.84 (m, 5H), 7.95–8.18 (m, 5H), 8.47 (1H, $J = 8.5$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₃₆H₃₅N₄O₅ m/z 603, (FAB⁺, nitrobenzyl alcohol) for m/z 127. Anal. (C₃₆H₃₅N₄O₅·1.5H₂O) C, H, I, N, S.

3-Ethyl-2-[[3-methyl-5-[2-(3-ethylbenzoxazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]benzothiazolium Iodide (29). This compound was synthesized from 3-ethyl-2-methylbenzoxazolinium *p*-toluenesulfonate and 3-methyl-4-oxothiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 10: UV-vis (MeOH) λ_{\max} 564.3 nm (ϵ 8.76 $\times 10^4$); ¹H-NMR (DMSO-*d*₆) δ 1.33–1.43 (m, 6H), 3.60 (s, 3H), 4.23 (q, $J = 7.1$ Hz, 2H), 4.72 (q, $J = 7.1$ Hz, 2H), 5.62 (d, $J = 13.2$ Hz, 1H), 6.70 (s, 1H), 7.28–7.42 (m, 2H), 7.53–7.75 (m, 4H), 7.96–8.08 (m, 2H), 8.24 (d, $J = 7.2$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₅H₂₄N₃O₅ m/z 462, (FAB⁺, nitrobenzyl alcohol) for m/z 127; HRMS (FAB⁺) for C₂₅H₂₄N₃O₅ calcd 462.1310, found 462.1309. Anal. (C₂₅H₂₄N₃O₅·1.75H₂O) C, I, N, S; H: calcd, 4.46; found, 3.92.

3-Ethyl-2-[[5-[2-(3-ethylbenzoxazolin-2-ylidene)ethylidene]-4-oxo-1,3-dithiolan-2-ylidene]methyl]benzothiazolium iodide (30). This compound was synthesized according to the reported method¹⁶ from 3-ethyl-2-methylbenzothiazolinium iodide and carbon disulfide: UV-vis (MeOH) λ_{\max} 586.8 nm (ϵ 6.54 $\times 10^4$); NMR studies (COSY and ROESY spectra (data are not shown)) revealed that compound 30 was a mixture (1:1) of geometrical isomers at the merocyanine moiety, ¹H-NMR (CDCl₃:DMSO-*d*₆ = 3.5:1.5) (one isomer) δ 1.47–1.55 (m, 6H), 4.33 (q, $J = 7.2$ Hz, 2H), 4.71–4.77 (m, 2H), 5.54 (d, $J = 13.5$ Hz, 1H), 7.38 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.43 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.49 (d, $J = 7.6$ Hz, 1H), 7.58 (d, $J = 7.6$ Hz, 1H), 7.68 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.83 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.84 (s, 1H), 8.00 (d, $J = 7.6$ Hz, 1H), 8.17 (d, $J = 13.5$ Hz, 1H), 8.25 (d, $J = 7.6$ Hz, 1H), (another isomer) δ 1.47–1.55 (m, 6H), 4.25 (q, $J = 7.2$ Hz, 2H), 4.71–4.77 (m, 2H), 5.39 (d, $J = 13.5$ Hz, 1H), 7.38 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.43 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.49 (d, $J = 7.6$ Hz, 1H), 7.58 (d, $J = 7.6$ Hz, 1H), 7.64 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.67 (s, 1H), 7.78 (dd, $J = 7.6, 7.6$ Hz, 1H), 7.95 (d, $J = 7.6$ Hz, 1H), 8.10 (d, $J = 13.5$ Hz, 1H), 8.20 (d, $J = 7.6$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₄H₂₁N₂O₂S₃ m/z 465, (FAB⁺, nitrobenzyl alcohol) for m/z 127; HRMS (FAB⁺) for C₂₄H₂₁N₂O₂S₃ calcd 465.0765, found 465.0765. Anal. (C₂₄H₂₁N₂O₂S₃·1.5H₂O) C, H, N, S; I: calcd, 20.48; found, 19.85.

3-Ethyl-2-[[3-ethyl-5-(3-methylbenzothiazolin-2-ylidene)-

4-oxothiazolidin-2-ylidene]methyl]benzothiazolium Acetate (31). A solution of 5 (4.2 g, 7 mmol) in a 0.1 M acetic acid solution (300 mL) of chloroform/methanol (1/2, v/v) was passed through the basic anion-exchange resin (about 120 mL, Diaion WA-21, acetate form), and the resin was washed with a 0.1 M acetic acid solution (200 mL) of methanol. The eluent was concentrated to about 50 mL, to which was added ethyl acetate (300 mL). The precipitate formed was collected and washed with ethyl acetate (50 mL) to give 31 (2.8 g, 67.4%) as orange crystals: UV-vis (MeOH) λ_{\max} 501.2 nm (ϵ 6.88 $\times 10^4$); ¹H-NMR (DMSO-*d*₆) δ 1.26 (t, $J = 7.1$ Hz, 3H), 1.37 (t, $J = 7.1$ Hz, 3H), 1.68 (s, 3H), 4.21 (s, 4.5H), 4.28 (q, $J = 7.2$ Hz, 2H), 4.70 (q, $J = 7.2$ Hz, 2H), 6.67 (s, 1H), 7.33 (t, $J = 7.7$ Hz, 1H), 7.50 (q, $J = 7.8$ Hz, 2H), 7.71 (q, $J = 8.5$ Hz, 2H), 7.94 (d, $J = 8.1$ Hz, 2H), 8.24 (d, $J = 8.1$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₇H₂₂N₃O₅ m/z 452, (FAB⁺, nitrobenzyl alcohol) for C₇H₇O₃S m/z 59; HRMS (FAB⁺) for C₂₇H₂₂N₃O₅ calcd 452.0925, found 452.0891. Anal. (C₂₇H₂₂N₃O₅·2.85H₂O·0.5C₂H₄O₂) C, S; H: calcd, 5.56; found, 4.98. N: calcd, 7.09; found, 6.43.

2-[[3-Cyclohexyl-5-(3-methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]-3-ethylbenzoxazolinium Chloride (32). This compound was synthesized from 2-(methylthio)benzothiazole and 4-oxo-3-cyclohexylthiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 5: UV-vis (MeOH) λ_{\max} 488.1 nm (ϵ 6.73 $\times 10^4$); ¹H-NMR (DMSO-*d*₆) δ 1.24–1.90 (m, 10H), 4.18 (s, 3H), 4.50 (q, $J = 7.0$ Hz, 2H), 4.60 (brs, 1H), 6.36 (s, 1H), 7.48 (dd, $J = 7.8, 7.8$ Hz, 1H), 7.48–7.61 (m, 3H), 7.79 (dd, $J = 8.0, 8.0$ Hz, 2H), 7.98 (dd, $J = 7.8, 7.8$ Hz, 2H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₇H₂₈N₃O₂S₂ m/z 490, (FAB⁺, nitrobenzyl alcohol) for Cl m/z 35. Anal. (C₂₇H₂₈ClN₃O₂S₂·2.5H₂O) C, H, Cl, N, O, S.

3-Ethyl-2-[[3-ethyl-5-[2-(3-ethyl-5-(trifluoromethyl)benzothiazolin-2-ylidene)ethylidene]-4-oxothiazolidin-2-ylidene]methyl]-4-methylthiazolium Iodide (33). This compound was synthesized from 3-ethyl-2-methyl-5-trifluorobenzothiazolium *p*-toluenesulfonate and 3-methyl-4-oxothiazolidine-2-thione in a manner analogous to the preparation of rhodacyanine dye 10: UV-vis (MeOH) λ_{\max} 559.2 nm (ϵ 8.39 $\times 10^4$); ¹H-NMR (CD₃OD) δ 1.32 (t, $J = 7.1$ Hz, 3H), 1.40 (t, $J = 7.1$ Hz, 3H), 1.45 (t, $J = 7.1$ Hz, 3H), 2.53 (s, 3H), 4.14 (q, $J = 7.1$ Hz, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 4.46 (q, $J = 7.2$ Hz, 2H), 5.87 (d, $J = 12.5$ Hz, 1H), 6.59 (s, 1H), 7.49 (s, 1H), 7.51 (d, $J = 8.9$ Hz, 1H), 7.65 (s, 1H), 7.84 (d, $J = 12.5$ Hz, 1H), 7.85 (d, $J = 8.9$ Hz, 1H); MS (FAB⁺, nitrobenzyl alcohol) for C₂₄H₂₅F₃N₃O₅ m/z 524, (FAB⁺, nitrobenzyl alcohol) for m/z 127; HRMS (FAB⁺) for C₂₄H₂₅F₃N₃O₅ calcd 524.1112, found 524.1108. Anal. (C₂₄H₂₅F₃N₃O₅·2H₂O) H, F, N, S; C: calcd, 4.25; found, 3.69. I: calcd, 18.46; found, 17.85.

X-ray Structure Analysis of 32 and 33. Compounds 32 and 33 were crystallized from ethanol and acetonitrile solutions, respectively. X-ray diffraction data were measured by a Rigaku AFC-5R instrument using Cu K α radiation and a graphite monochromometer. Structures were determined by direct-method SHELXS86 and successive Fourier syntheses and refined by a full-matrix least-squares method. Full crystallographic details are available as Supporting Information.

In Vitro Clonogenic Assay. CX-1 and CV-1 cell lines were grown in a 50:50 mix of Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% calf serum (Hyclone Laboratories Inc., Logan, UT) and antibiotics at 37 °C under 5% CO₂, 95% air, and 100% humidity. CV-1 (normal African green monkey kidney) was obtained from the American Type Culture Collection (Rockville, MD), and CX-1 (human colon carcinoma) from Dr. M. Wolpert (National Cancer Institute).

For the clonogenic assay, cells were seeded at 1500 cells/wells for CX-1 and 1000 cells/wells for CV-1 in 96-well plates (Becton Dickinson Labware, Lincoln Park, NJ). The assay was performed in duplicate. The drugs were first dissolved in dimethyl sulfoxide, to prepare 10 mg/mL stock solutions. The final drug solution was made by mixing 100 μ L of this stock solution with 10 mL of 5% CS DME media solution. On the

following day, cells were treated with test compounds at varied concentrations and cultured precisely for 24 h in the media. After rinsing, cells were incubated in drug-free medium for 2 weeks. Colonies were stained with 2% crystal violet in 70% ethanol and counted by an automated colony counter (Artek counter model 880, Dynatech Laboratories Inc., Chantilly, VA).

In Vivo Human Tumor Xenografts in Nude Mice. Male Swiss nu/nu mice (about 5 weeks old) were obtained from Taconic Farm, Inc. (Germantown, NY). Group housing (5/cage) was provided in polycarbonate cages with a wire top and filters. Mice were allowed to acclimate for 1 week prior to experiments. Only normal, healthy mice were used. Human melanoma LOX cells used in this model were first grown *in vitro* in nude mice. On the day of *in vivo* implantation, tumors were excised and a single cell suspension was prepared. RBCs were lysed by ammonium chloride. Each mouse received 2×10^6 LOX cells (trypan blue-negative) in 0.2 mL of PBS by *in vivo* injection. Test compounds (0.2 mL/20 g of mouse body weight, 45% encapsin HPB (hydroxypropyl β -cyclodextrin; American Maise-Products Co.) in water) were administered *in vivo* for LOX tumor-bearing mice with appropriately determined doses and schedules. Evaluation against human ovarian carcinoma OVCARIII cells and human colon carcinoma CX-1 cells was performed in a manner analogous to the LOX *in vivo* tumor model.

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Supporting Information Available: X-ray crystallographic data for compounds 32 and 33 (21 pages). Ordering information is given on any current masthead page.

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